Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE NECESSITY OF A STANDARD BLOOD-AGAR PLATE FOR THE DETERMINATION OF HEMOLYSIS BY STREPTOCOCCI*

WESLEY C. BECKER

From the Memorial Institute for Infectious Diseases, Chicago

The manner of growth of streptococci on blood-agar plates, as first suggested by Schottmüller, has been found a valuable means of differentiation. Schottmüller[1] found that by growth on a mixture composed of 2 c.c. of defibrinated human blood and 5 c.c. of plain agar, streptococci can be differentiated into 3 groups, which he describes as follows: Streptococcus longus seu erysipelatos, which appears after from 18 to 24 hours' incubation as a grayish circular colony surrounded by a zone of hemolysis from 2 to 3 mm. in diameter; Streptococcus mitior seu viridans, which appears after 24 hours' incubation as a very small greenish colony, no hemolysis being present except where the medium is very thin; and Streptococcus mucosus, which appears after 24 hours' incubation as a mucoid greenish very moist growth. These results have been investigated by a great number of workers and Schumacher,[2] Silverstrom,[3] Mandelbaum[4] and many others have accepted the method in general as a valuable means of differentiation. On the other hand Bietske and Rosenthal[5] claimed that hemolytic strains could be changed into nonhemolytic and vice versa. Mandelbaum[4] ascribes the different results to the use of different media. He found that on blood-agar plates made according to the formula of Schottmüller a type of hemolysis is produced by Streptococcus mitior which he describes as follows: "hemolytic zone in mitior appears rarely in 24 hours, most often at 48 to 96 hours' incubation; microscopically one finds blood cells directly beneath the colony, while many discolored cells remain in the clear zone becoming more abundant further away from the colony." Smith and Brown[6] have called atten-

---

* Received for publication August 5, 1916.

[1] München. Med. Wchnschr., 1903, 50, p. 849.

[2] Centralbl. f. Bakteriol., I, O., 1906, 41, pp. 628, 712.

[3] Ibid., p. 409.

[4] Ztschr. f. Hyg., u. Infektionskrankh., 1908, 58, p. 26.

[5] Arb. a. d. path. Inst., Berlin, 1906.

[6] Jour. Med. Research, 1914, 31, p. 455.

tion to a similar variation in hemolysis and have designated the distinct hemolysis as Type Beta and the indistinct as Type Alpha.

Little attention seems to have been paid to Mandelbaum's work in this country. Recorded results in the determination of hemolysis on blood agar are unsatisfactory and difficult of comparison. Defibrinated and citrated human, horse, rabbit, sheep, and goat blood in varying amounts are used in the preparation of blood-agar plates and the results are read after from 24 to 96 hours' incubation. Von Lingelsheim[7] has called attention to the necessity of a standard technic in the determination of hemolysis, and more recently Holman[8] has proposed a medium composed of 5 c.c. of defibrinated human blood to 100 c.c. of plain agar. Holman also directs attention to variations in hemolysis due to other factors, as period of incubation, salt concentration of the agar base, temperature of the agar to which the blood is added, and the age of the blood-agar plates. It is evident that in order to secure data of at all comparable significance a standard blood-agar plate must be used.

In the work here reported, 5 strains of streptococci were studied on blood-agar plates of varying composition.

Organism 1 was a strongly hemolytic long-chained streptococcus isolated from the skin of a case of erysipelas; Organism 2 was a hemolytic strain from a scarlatinal sore throat; Organism 3 was a long-chained hemolytic streptococcus obtained from the tonsils in a case of rheumatic fever; Organism 4 was a nonhemolytic short-chained streptococcus from the sputum in a case of pneumonia; Organism 5 was a green-producing streptococcus obtained from the same tonsils as 3. Organisms 1 and 5 fermented saccharose, lactose, and maltose; Organism 2 fermented lactose, mannite, raffinose, maltose, and dextrose; Organism 3 fermented saccharose, lactose, maltose, and dextrose; Organism 4 fermented maltose only.

Each of these organisms was repeatedly transferred from a single colony on human defibrinated blood agar plates to ascites dextrose broth and again plated out in order to insure a pure strain. The inoculation was made by streaking a drop of a 4- to 6-hour ascites-dextrose-broth culture on the freshly prepared plates. The plates were studied after 24 hours' incubation at 37 C., after 48 hours, and after 96 hours. The fundamental conditions were kept as nearly uniform as possible. Thus, all the agar used was of one lot; a portion was made 1% alkaline with sodium hydrate, another portion 1% acid with hydrochloric acid, and a third portion of standard agar was made up with 1% dextrose. Blood was used unless otherwise mentioned in amounts of 1 c.c. of defibrinated blood or 2 c.c. of citrated blood to 6 c.c. of standard agar. Citrated blood was blood collected by aspiration from a vein into an equal quantity of 2% sodium citrate in physiologic salt solution.

---

[7] Kolle and Wassermann, Handb. d. pathogen. Mikroorganismen, 1912, 4, p. 463.

[8] Jour. Med. Research, 1916, 34, p. 377.

The observations with respect to hemolysis may be grouped under the following heads: (1) kind of medium; (2) kind of blood; (3) method of collecting blood; (4) amount of blood per plate; (5) number of colonies per plate; (6) period of incubation.

Kind of Medium.—Marked variations both macroscopically and microscopically occurred in the hemolytic zone on plain agar, alkaline agar, acid agar, and dextrose agar, to which blood in the proportion before mentioned had been added. Thus, no hemolysis was produced by any of the organisms in alkaline blood agar. Organism 1 produced a distinct hemolytic zone on plain human-defibrinated-blood agar, but an indistinct zone on the acid medium. With Organism 3, the results were the reverse. Organism 2 produced a distinct zone of hemolysis on plain defibrinated-sheep-blood agar and plain citrated-sheep-blood agar and on acid defibrinated-sheep-blood agar, but an indistinct zone of hemolysis on acid citrated-sheep-blood agar. Similar variations occurred in the case of other organisms. Variations in the width of the hemolytic zone also occurred. These irregularities did not appear to be dependent on the luxuriousness of the growth. Hemolysis was distinctly inhibited on dextrose blood agar, as has been shown by Lyall[10] and others. Organism 5 (viridans) gave practically equal amounts of greenish coloration on plain, acid, and alkaline agar, and more pronounced coloration on dextrose blood agar. Organism 2 produced a greenish color on dextrose defibrinated-human-blood agar, while no change was produced by Organisms 1, 3, 4, and 5. This, which is somewhat contradictory to the observations of Ruediger,[9] emphasizes the wide variations in this group.

Kind of Blood.—Blood of various species has been used, tho only a few comparative studies have been made. Silverstrom used defibrinated rabbit, horse, and human blood, but he mentions no marked differences. Guinea-pig blood has been reported by a number of observers[7] as unsuitable because of the rapid distintegration of the corpuscles. Kerner[11] found that the corpuscles of the dog are most easily hemolyzed, while human and frog corpuscles are the most resistant. In this study sheep, goat, horse, rabbit, and human bloods were used. Variations are especially marked in the microscopic appearance of the hemolytic zone. Organism 1 in 24 hours' incubation totally dissolved on a plain-agar base defibrinated sheep and horse corpuscles, but did not dissolve goat, rabbit, or human corpuscles. Organism 2 dissolved citrated sheep corpuscles in acid agar, but not defibrinated sheep corpuscles in either acid or plain agar. Human cells were dissolved in acid media, while rabbit corpuscles were dissolved in plain agar, and horse corpuscles were not dissolved in any media. Organism 3 dissolved rabbit corpuscles only. Organism 4 produced hemolysis on horse blood only. Green-production by Organism 5 was most pronounced on sheep blood and least on human blood. Methemoglobin formed most quickly on rabbit blood.

---

[9] Jour. Infect. Dis., 1906, 3, p. 663.

[10] Jour. Med. Research, 1914, 30, p. 515.

[11] Centralbl. f. Bakteriol., I. O., 1905, 38, p. 223.

Method of Collecting Blood.—Defibrinated or citrated blood is used by the majority of workers. Schumaker used defibrinated goat and human blood, which had been prevented from clotting by means of hirubin. He found no differences. Bernstein and Epstein[12] used ox blood, secured as follows: 400 c.c. of ox blood were collected in a sterile flask containing 30 c.c. of 1% ammonium oxalate in distilled water and 0.5 c.c. of 40% formalin. It was allowed to stand one-half hour, after which it was diluted with equal parts of 0.9% salt solution. Schottelius advised the use of fresh human blood, but gave no reason for its use. Citrated and defibrinated blood from rabbit, sheep, and man were used in this study. No striking macroscopic differences were observed. Microscopically, the cells did not dissolve as readily in citrated-blood agar as in defibrinated-blood agar. No regularity in this regard can be determined, however.

Amount of Blood per Plate.—Perhaps the most striking variations were seen with the use of various amounts of blood. Organism 1 produced a wider zone on medium containing 3 and 6 drops of human defibrinated blood than on that containing 9 and 12 drops. In the plates made with 3 and 6 drops the hemolysis was distinct, while on those made with 9 and 12 drops the hemolysis was indistinct. Organism 2 produced a distinct zone of hemolysis from 10 to 14 mm. wide on medium containing 3 and 6 drops of human defibrinated blood and a zone from 4 to 8 mm. wide on that containing 9 drops, while on medium containing 12 drops of blood it produced in 24 hours an indistinct zone of hemolysis from 4 to 8 mm. wide. Organism 3 produced a distinct zone of hemolysis from 1 to 3 mm. wide on plates containing 3, 6, and 9 drops of blood, while on the plate containing 12 drops of human defibrinated blood it produced indistinct hemolysis in a zone from 3 to 6 mm. in extent.

---

[12] Jour. Infect. Dis., 1906, 3, p. 772.

Number of Colonies per Plate.—On various occasions it was noticed that hemolysis was clear and distinct in case of isolated colonies, while indistinct and microscopically very different when the colonies were closely grouped. The reverse condition was noticed with other strains. In the case of S. viridans, green-production occurred in the form of a distinct narrow greenish ring when the colonies were isolated, while a diffuse discoloration occurred when the colonies were close together.

Period of Incubation.—Plates were read after 24, 48, and 96 hours' incubation at 37.5 C. In some cases the cells were dissolved after 48 hours when they were present at 24 hours. In the case of Organism 2 the distinct hemolytic zone at 24 hours' incubation, after 48 hours' incubation was totally obscured on rabbit and horse blood, and was indistinct on sheep and human blood because of the production of methemoglobin. The greenish coloration of S. viridans was usually more distinct at 48 hours' incubation, tho on rabbit blood a brownish color, instead of green, was produced at 96 hours' incubation. Organism 4 produced on horse blood at 96 hours' incubation a greenish zone indistinguishable from the colonies and plates of Organism 5 (viridans).

DISCUSSION

In the determination of a standard medium certain points must be kept in mind: (1) availability, (2) constancy of material, and (3) the necessary elements for proper growth of the organism. While this paper was under process of completion, Holman recommended as a standard medium for the determination of hemolysis, 5 c.c. of defibrinated human blood to 100 c.c. of agar, made up according to certain directions which he gives. As a standard agar has been used in water analysis for several years and is used in most laboratories in this country, it is hardly necessary to have a specific plain agar base, tho the agar base suggested by Holman is much easier to make and would probably serve the purpose. Human blood is the most readily available and resists hemolysis by weakly hemolytic strains the best. I believe the Holman medium contains too small a quantity of blood, the hemolysis appearing indistinct in many cases. Many freshly isolated strains grow very poorly on medium containing such a small amount of hemoglobin. Schottmüller originally used 2 c.c. of defibrinated human blood to 5 c.c. of agar. This I believe to be more blood than is necessary and the results are not sufficiently superior to warrant its use.

The following technic for the preparation of a standard blood agar is suggested: Standard agar used in water analysis is placed in quantities of from 40 to 100 c.c. in flasks. These are heated until the agar is melted, and then cooled to 45-50 C. One cubic centimeter of defibrinated human blood is added for each 6 c.c. of the agar base and the whole thoroughly mixed. Approximately 7 c.c. are used for each plate. Surface streaks are made and results noted after 24 hours' incubation at 37 C. Isolated colonies only should be described.

On such plates, strains of streptococci, pneumococci, and gonococci can usually be readily grown and distinguished.

CONCLUSIONS

Marked variations occur in both the macroscopic and microscopic appearance of the hemolytic zone of streptococci on blood-agar plates.

Differentiation and comparison of strains of streptococci on blood agar of indefinite or varying composition is wholly unreliable and valueless. For this purpose a standard blood agar, such as that recommended in this article, should be used.